

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application.

1.-30. (Cancelled)

31. (Currently Amended) A cultured skin construct having at least two layers, comprising:

(a) a first layer of cultured dermal fibroblast cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin;
- (iii) fibronectin,
- (iv) tenascin; and,
- (v) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of **both** exogenous matrix components **and a mesh member** during the culturing conditions; and,

(b) a second layer of keratinocyte cells disposed on the first layer to form an epidermal cell layer, wherein the epidermal cell layer is multilayered, stratified, differentiated and exhibits a basal layer, suprabasal layer, a granular layer and a stratum corneum;

and wherein the bilayered cultured skin construct has a basement membrane present at the junction of the first and second layers.

32. (Previously Presented) The cultured skin construct of claim 31, wherein said cultured cells are cultured in chemically defined media.

33. (Previously Presented) The cultured skin construct of claim 31, wherein said first layer has cultured cells from dermal papilla of hair follicles localized on said first layer.

34. (Previously Presented) The cultured skin construct of claim 31, wherein the cultured dermal fibroblast cells are genetically modified to produce extracellular matrix components.

35. (Previously Presented) The cultured skin construct of claim 31, wherein the cultured dermal fibroblast cells are genetically modified to produce a growth factor, hormone, peptide, or protein.

36. (Previously Presented) The cultured skin construct of any of claims 31-35, wherein the construct is cohesive in having physical unitary integrity.

37. (Currently Amended) A cultured skin construct having at least two layers, comprising:

(a) a first layer of cultured dermal fibroblast cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin;
- (iii) fibronectin,
- (iv) tenascin; and,
- (v) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of **both** exogenous matrix components **and a mesh member** during the culturing conditions; and,

(b) a second layer of cells comprising epithelial cells disposed on the first layer.

38. (Previously Presented) The cultured skin construct of claim 37, wherein the epithelial cells are selected from the group consisting of keratinocytes, corneal epithelial cells, epithelial cells from oral mucosa, esophageal epithelial cells, and uroepithelial cells.

39. (Currently Amended) A cultured tissue construct having at least three layers, comprising:

(a) a first layer of cultured fibroblasts cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin; and,
- (iii) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of both exogenous matrix components and a mesh member during the culturing conditions;

(b) a second layer of cells comprising epithelial cells disposed on the first layer; and,

(c) a third layer of cells disposed on the second layer of epithelial cells.

40. (Previously Presented) The cultured tissue construct of claim 39, wherein said fibroblast cells contained within said first layer are derived from tissue selected from the group consisting of neonate male foreskin, dermis, tendon, lung, cartilage, urethra, corneal stroma, oral mucosa, umbilical cord, and intestine.

41. (Previously Presented) The cultured tissue construct of claim 39, wherein said fibroblast cells contained within said first layer are dermal fibroblasts.

42. (Previously Presented) The cultured tissue construct of claim 39, wherein said first layer has cultured cells from dermal papilla of hair follicles localized on said first layer.

43. (Previously Presented) The cultured tissue construct of claim 39, wherein said cultured cells are cultured in chemically defined media.

44. (Previously Presented) The cultured tissue construct of claim 39, wherein the cultured fibroblast cells are genetically modified to produce extracellular matrix components.

45. (Previously Presented) The cultured tissue construct of claim 39, wherein the cultured fibroblast cells are genetically modified to produce a growth factor, hormone, peptide, or protein.

46. (Previously Presented) The cultured tissue construct of claim 39, wherein the epithelial cells are selected from the group consisting of keratinocytes, corneal epithelial cells, epithelial cells from oral mucosa, esophageal epithelial cells, and uroepithelial cells.

47. (Previously Presented) The cultured tissue construct of any of claims 39-46, wherein the construct is cohesive in having physical unitary integrity.

48. (Currently Amended) A method for producing a cultured tissue construct, comprising,

(a) seeding fibroblast cells capable of synthesizing an extracellular matrix on a porous membrane in a culture vessel in a cell culture medium at about 80% to about 100% confluence;

(b) stimulating the fibroblast cells to synthesize, secrete and organize extracellular matrix components; and,

(c) continued culturing of the fibroblast cells until the cells form a layer of synthesized extracellular matrix of at least about 30 microns thick, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) tenascin; and,
- (iii) glycosaminoglycans;

and wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of both exogenous matrix components and a mesh member during the culturing conditions.

49. (Previously Presented) The method of claim 48, wherein the fibroblast cells are derived from tissue selected from the group consisting of neonate male foreskin, dermis, tendon, lung, cartilage, urethra, corneal stroma, oral mucosa, umbilical cord, and intestine.

50. (Currently Amended) A method for transplantation or implantation of a cultured skin construct into a patient comprising transplanting or implanting into a patient, a cultured skin construct having at least two layers, comprising:

(a) a first layer of cultured dermal fibroblast cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin;
- (iii) fibronectin,
- (iv) tenascin; and,
- (v) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of **both** exogenous matrix components **and a mesh member** during the culturing conditions; and,

(b) a second layer of keratinocyte cells disposed on the first layer to form an epidermal cell layer, wherein the epidermal cell layer is multilayered, stratified, differentiated and exhibits a basal layer, suprabasal layer, a granular layer and a stratum corneum;

and wherein the bilayered cultured skin construct has a basement membrane present at the junction of the first and second layers.

51. (Currently Amended) A method for transplantation or implantation of a cultured skin construct into a patient comprising transplanting or implanting into a patient, a cultured skin construct having at least two layers, comprising:

(a) a first layer of cultured dermal fibroblast cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin;
- (iii) fibronectin,
- (iv) tenascin; and,
- (v) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of **both** exogenous matrix components **and a mesh member** during the culturing conditions; and,

(b) a second layer of cells comprising epithelial cells disposed on the first layer.

52. (Currently Amended) A method for transplantation or implantation of a cultured tissue construct into a patient comprising transplanting or implanting into a patient, a cultured tissue construct having at least three layers, comprising:

(a) a first layer of cultured fibroblasts cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin; and,
- (iii) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of **both** exogenous matrix components **and a mesh member** during the culturing conditions;

(b) a second layer of cells comprising epithelial cells disposed on the first layer; and,

(c) a third layer of cells disposed on the second layer of epithelial cells.

53. (Previously Presented) The cultured skin construct of claims 31 or 37, wherein the fibroblast cells are cultured in a matrix production medium to produce the layer of extracellular matrix.

54. (Previously Presented) The cultured skin construct of claim 53, wherein the matrix production medium comprises an ascorbate derivative.

55. (Previously Presented) The cultured skin construct of claim 54, wherein the ascorbate derivative is selected from the group consisting of sodium ascorbate, ascorbic acid or a derivative thereof.

56. (Previously Presented) The cultured skin construct of claim 54, wherein the matrix production medium further comprises epidermal growth factor,

hydrocortisone, ethanolamine, o-phosphoryl-ethanolamine, insulin, transferring, triiodothyronine, selenium, L-ascorbic acid, L-proline, glycine or combinations thereof.

57. (Previously Presented) The cultured tissue construct of claim 39, wherein the fibroblast cells are cultured in a matrix production medium to produce the layer of extracellular matrix.

58. (Previously Presented) The cultured tissue construct of claim 57, wherein the matrix production medium comprises an ascorbate derivative.

59. (Previously Presented) The cultured tissue construct of claim 58, wherein the ascorbate derivative is selected from the group consisting of sodium ascorbate, ascorbic acid or a derivative thereof.

60. (Previously Presented) The cultured tissue construct of claim 58, wherein the matrix production medium further comprises epidermal growth factor, hydrocortisone, ethanolamine, o-phosphoryl-ethanolamine, insulin, transferring, triiodothyronine, selenium, L-ascorbic acid, L-proline, glycine or combinations thereof.

61. (Previously Presented) The method of claim 48, wherein stimulating the fibroblast cells comprises culturing them in a matrix production medium to produce the layer of extracellular matrix.

62. (Previously Presented) The method of claim 61, wherein the matrix production medium comprises an ascorbate derivative.

63. (Previously Presented) The method of claim 62, wherein the ascorbate derivative is selected from the group consisting of sodium ascorbate, ascorbic acid or a derivative thereof.

64. (Previously Presented) The method of claim 62, wherein the matrix production medium further comprises epidermal growth factor, hydrocortisone, ethanolamine, o-phosphoryl-ethanolamine, insulin, transferring, triiodothyronine, selenium, L-ascorbic acid, L-proline, glycine or combinations thereof.

65. (New) A cultured skin construct having at least two layers, comprising:

(a) a first layer of cultured dermal fibroblast cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells



on one surface of a porous membrane, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin;
- (iii) fibronectin,
- (iv) tenascin; and,
- (v) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of exogenous matrix components during the culturing conditions; and,

(b) a second layer of keratinocyte cells disposed on the first layer to form an epidermal cell layer, wherein the epidermal cell layer is multilayered, stratified, differentiated and exhibits a basal layer, suprabasal layer, a granular layer and a stratum corneum;

and wherein the bilayered cultured skin construct has a basement membrane present at the junction of the first and second layers.

66. (New) A cultured skin construct having at least two layers, comprising:

(a) a first layer of cultured dermal fibroblast cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells on one surface of a porous membrane, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin;
- (iii) fibronectin,

- (iv) tenascin; and,
- (v) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of exogenous matrix components during the culturing conditions; and,

(b) a second layer of cells comprising epithelial cells disposed on the first layer.

67. (New) A cultured tissue construct having at least three layers, comprising:

(a) a first layer of cultured fibroblasts cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells on one surface of a porous membrane, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin; and,
- (iii) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components during the culturing conditions;

(b) a second layer of cells comprising epithelial cells disposed on the first layer; and,

(c) a third layer of cells disposed on the second layer of epithelial cells.

68. (New) A method for producing a cultured tissue construct, comprising,

(a) seeding fibroblast cells capable of synthesizing an extracellular matrix on a porous membrane in a culture vessel in a cell culture medium at about 80% to about 100% confluence;

(b) stimulating the fibroblast cells to synthesize, secrete and organize extracellular matrix components; and,

(c) continued culturing of the fibroblast cells until the cells form a layer of synthesized extracellular matrix of at least about 30 microns thick, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) tenascin; and,

(iii) glycosaminoglycans;

and wherein said extracellular matrix is produced by the cultured fibroblast cells on one surface of a porous membrane in the absence of exogenous matrix components during the culturing conditions.

69. (New) A method for transplantation or implantation of a cultured skin construct into a patient comprising transplanting or implanting into a patient, a cultured skin construct having at least two layers, comprising:

(a) a first layer of cultured dermal fibroblast cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells on one surface of a porous membrane, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) decorin;

(iii) fibronectin,

(iv) tenascin; and,

(v) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of exogenous matrix components during the culturing conditions; and,

(b) a second layer of keratinocyte cells disposed on the first layer to form an epidermal cell layer, wherein the epidermal cell layer is multilayered, stratified, differentiated and exhibits a basal layer, suprabasal layer, a granular layer and a stratum corneum;

and wherein the bilayered cultured skin construct has a basement membrane present at the junction of the first and second layers.

70. (New) A method for transplantation or implantation of a cultured skin construct into a patient comprising transplanting or implanting into a patient, a cultured skin construct having at least two layers, comprising:

(a) a first layer of cultured dermal fibroblast cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells on one surface of a porous membrane, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin;
- (iii) fibronectin,
- (iv) tenascin; and,
- (v) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of exogenous matrix components during the culturing conditions; and,

(b) a second layer of cells comprising epithelial cells disposed on the first layer.

71. (New) A method for transplantation or implantation of a cultured tissue construct into a patient comprising transplanting or implanting into a patient, a cultured tissue construct having at least three layers, comprising:

(a) a first layer of cultured fibroblasts cells which produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells on one surface of a porous membrane, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin; and,
- (iii) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components during the culturing conditions;

- (b) a second layer of cells comprising epithelial cells disposed on the first layer; and,
- (c) a third layer of cells disposed on the second layer of epithelial cells.